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*RESEARCH REPORT 76*

# FOOD HABITS OF STRIPED MARLIN AND SAILFISH OFF MEXICO AND SOUTHERN CALIFORNIA

*RESEARCH REPORT 77*

# GENIC POLYMORPHISM OF BLOOD PROTEINS FROM WHITE MARLIN

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By Douglas H. Evans and Paul G. Wares

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By Philip H. Edmunds

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By Douglas H. Evans and Paul G. Wares

RESEARCH REPORT 76

UNITED STATES DEPARTMENT OF THE INTERIOR

Rogers C. B. Morton, *Secretary*

Nathaniel P. Reed, *Assistant Secretary for Fish and Wildlife and Parks*

FISH AND WILDLIFE SERVICE

Bureau of Sport Fisheries and Wildlife

Spencer H. Smith, *Director* (Acting)

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## ABSTRACT

Stomach contents of 924 striped marlin (*Tetrapturus audax*) landed in the sport catches at Mazatlán, Sinaloa, and Buena Vista, Baja California Sur, Mexico, and San Diego, Calif., and of 197 sailfish (*Istiophorus platypterus*) from Mazatlán and Buena Vista were examined. The striped marlin and sailfish fed primarily on pelagic fishes and cephalopods. By volume the major foods were squid (principally *Dosidicus gigas*) for striped marlin at Mazatlán and for striped marlin and sailfish at Buena Vista, northern anchovy (*Engraulis mordax*) for striped marlin at San Diego, and threadfin (*Polydactylus* sp.) for sailfish at Mazatlán. Locality differences in food habits were pronounced, and some seasonal and yearly differences were found.

# FOOD HABITS OF STRIPED MARLIN AND SAILFISH OFF MEXICO AND SOUTHERN CALIFORNIA

**By Douglas H. Evans and Paul G. Wares, *Fishery Biologists***
Tiburon Fisheries Laboratory, Tiburon, California

In 1967 the Tiburon Fisheries Laboratory initiated a study of the life histories of marlins and sailfish in the eastern Pacific Ocean. One phase of the program was the analysis of the stomach contents of specimens landed in the sport catch in three popular fishing areas. Sailfish (*Istiophorus platypterus*) were examined from two localities in Mexico: Mazatlán, Sinaloa (Lat. 23°12′N, Long. 106°28′W), and Buena Vista, Baja California Sur (Lat. 23°39′N, Long. 109°41′W), both near the mouth of the Gulf of California. Striped marlin (*Tetrapturus audax*) were sampled from these two localities and from San Diego, Calif., (Lat 32°40′N, Long. 117°14′W). Some blue marlin (*Makaira nigricans*) and fewer black marlin (M. *indica*) are taken in the sport catch at Mazatlán and Buena Vista but neither species is taken off California. Food of these species was not studied. Nearly all of the angling for billfish in the two Mexican locations is done by United States citizens.

The striped marlin fishing off San Diego occurs from July through November, the bulk of the catch generally being made from late August through early October. Fishing seasons at the Mexican localities are not well defined, and some billfish are taken year-round. The fishing off Mazatlán is best for striped marlin from December through May and for sailfish from April through September. At Buena Vista the striped marlin fishing is most productive from March through July. Fishing for sailfish is best there from June through November but never atains the success of striped marlin fishing. The differences in temporal distribution of the two species between the Mexican study areas, 180 miles apart on opposite sides of the Gulf, may be partly related to temperature patterns as the sea surface is as much as 4°C warmer (LaViolette and Seim, 1969) on the Mazatlán side from May through August. The area near the entrance to the Gulf is a complex zone where water from the Gulf interacts with the California Current and subtropical water (Griffiths, 1968). The general direction of circulation in the Gulf is counterclockwise.

In using the name *I. platypterus* for the sailfish we have followed the recent revision by Morrow and Harbo (1969),

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NOTE.—The Tiburon Fisheries Laboratory was transferred in October 1970 from the Bureau of Sport Fisheries and Wildlife, U.S. Fish and Wildlife Service, Department of the Interior, to the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Department of Commerce.

who regard the genus *Istiophorus* as monotypic with a worldwide distribution in warm and temperate seas. The striped marlin occurs only in the Pacific and Indian Oceans.

We give special thanks to persons who permitted us to examine specimens for our studies: Col. Eugene Walters, proprietor of Rancho Buena Vista; Mr. Bill Heimpel, proprietor of the Star Fleet at Mazatlán; and Mrs. Lois Ibey, Secretary of the San Diego Marlin Club. Their cooperation and that of the individual anglers made possible this study as well as continuing phases of our program. Larry Coe, Howard Ness, and Dan Eilers assisted in the collection of data. Drs. Richard Rosenblatt, W. I. Follett, William N. Eschmeyer, and Edmund S. Hobson, and Pearl M. Sonoda assisted with the identification of fishes. Dustin D. Chivers, John H. Wormuth, and Dr. John A. McGowan assisted with the identification of invertebrates. We also wish to thank William N. Eschmeyer and Lillian Dempster for critically reviewing the manuscript.

## METHODS

Most of the fish examined were caught within 25 miles of San Diego or within 10 to 20 miles of the Mexican ports at which they were landed. Stomach samples were obtained usually within 4 to 6 hours after capture. Stomachs from a total of 924 striped marlin and 197 sailfish were examined. Stomachs that were everted or that contained only fluid were regarded as empty. The number of empty stomachs was not recorded in the 1967 data from Mexico. Dates of sampling and numbers of striped marlin stomachs examined at each location were as follows: Mazatlán—4/10-5/6/67, 57 stomachs; 4/11-5/23/68, 105 stomachs (82 or 78% with food); 12/4-12/11/68, 4 stomachs (all with food); Buena Vista—5/18-6/5/67, 29 stomachs; 4/18-7/16/68, 392 stomachs (274 or 70% with food); San Diego—8/16-9/24/67, 177 stomachs (116 or 66% (132 or 83% with food). Sailfish stomachs were examined as follows: Mazatlán with food); 8/30-10/25/68, 160 stomachs—4/16-5/7/67, 41 stomachs; 4/13-6/9/68, 130 stomachs (105 or 81% with food); 12/4-12/11/68, 5 stomachs (all with food): Buena Vista—4/27-7/16/68, 21 stomachs (14 or 67% with food). In addition, during 1969, 600 striped marlin and 50 sailfish stomachs were examined in Mexico for the occurrence of prey species not found the previous 2 years. Average weights of the striped marlin examined from Mexican localities were 103 pounds in 1967 and 107 pounds in 1968. San Diego marlin averaged 119 pounds in 1967 and 134 pounds in 1968. Sailfish averaged 76 pounds in 1967 and 79 pounds in 1968.

Food items were preserved in formalin and later sorted, identified to the lowest taxon possible, and counted. The volume in fluid ounces of each taxonomic group was measured by water displacement, except for the 1967 samples from San Diego, for which only the total volume of the contents of each stomach was measured. Baits (principally flying fish, mullets, sardines, and mackerels) were identifiable by the mark of the hook or by the presence of thread used to harness the bait and were not included in the analysis. We have no estimate of the extent to which partial or complete regurgitation of stomach contents may have occurred.

## RESULTS

Food for striped marlin and sailfish consisted of invertebrates and fishes (table 1). The invertebrates most frequently eaten were various squids and the paper nautilus (*Argonauta* sp.); others occurred occasionally in trace amounts. The digested condition of most

of the squid precluded identification, but, several intact specimens were retained for study. Of these, *Loligo opalescens* was identified from the stomachs of San Diego striped marlin. *Dosidicus gigas* was common in the material from the two areas in Mexico. One specimen each of *Symplectoteuthis luminosa* (from a striped marlin) and *Thelidoteuthis alessandrini* (from a sailfish) was identified from the Buena Vista material. At least 38 species of fishes representing 23 families occurred in stomochs of striped marlin from the three study areas. Sailfish stomachs contained at least 24 species of fishes (15 families). All of the fishes were teleosts, with the exception of two occurrences of small thresher sharks (*Alopias vulpinus*) in San Diego striped marlin.

Many of the stomachs had ulcers. These were in varying stages from perforated ulcers to ones healed over with mounds of scare tissue. We have not made a histological analysis of them, but on several occasions anglers at the scene who were medical doctors remarked that they appeared similiar to human gastric ulcers. Of 563 striped marlin stomachs checked in 1968, 14% had ulcers; of 151 sailfish stomachs 22% had ulcers. We suspect that injuries from the spines of prey species are a factor in the ulceration of the stomach. The dorsal spines of triggerfishes especially might cause such injuries. The istiophorid stomach is a blind pouch with an opening only at the anterior end, and many of the ulcers were located at the distal end of the stomach, where squid beaks and bony debris tend to accumulate. Iversen and Kelly (in press) have found ulcers in blue and black marlin from Hawaiian waters. They reported a lower occurrence rate (8%) for blue marlin than we found for striped marlin or sailfish and reported the presence of nematodes in several of the ulcers.

The data were examined for differences in food habits between the species, between sexes, between localities, and with time. Pronounced differences were found between the species and locations and with time. The data are therefore reported separately for the two species, and locality, seasonal, and yearly differences are discussed for each. Variations due to size and sex differences were minor, so the data for all sizes and both sexes were combined. A preliminary inspection of the data suggested a periodicity in total food volumes and average volumes of squid per stomach. However, we looked for possible correlations between these two variables and the lunar cycle but found no consistent correlation in either.

## STRIPED MARLIN

### Locality differences

At San Diego the bulk of the diet was composed of three species of fish which do not range into the Mexican study areas. These species are the northern anchovy (*Engraulis mordax*), the Jack mackerel (*Trachurus symmetricus*), and the Pacific saury (*Cololabis saira*), which respectively occurred in 90%, 31%, and 15% of the stomachs with food. Squid were found in 54% and 69% of the stomachs at Mazatlán and Buena Vista, respectively, but in only 4% at San Diego. The round herring (*Etrumeus teres*) occurred in 27% of the Buena Vista stomachs but in none of the stomachs at Mazatlán or San Diego, although both these regions are within its range. The paper nautilus, triggerfish (*Balistes* sp.), and threadfin (*Polydactylus* sp.) were found at Mazatlán (17%, 8%, and 6%, respectively) but not at Buena Vista or San Diego. The chub mackerel (*Scomber japonicus*) and the Mexican scad (*Decapterus hypodus*) were more common in striped marlin from Buena Vista than from Mazatlán, whereas cornetfish (*Fistularia* sp.) and several other species occurred more frequently in the Mazatlán samples (table 1).

TABLE 1.—*Percent occurrence of prey species in stomachs of striped marlin and sailfish sampled at San Diego, Mazatlán, and Buena Vista in 1967 and 1968*

[Data are based on the number of stomachs which contained food; X denotes species noted during qualitative observations in 1969 at the Mexican localities; T denotes trace occurrence]

| | Striped marlin | | | Sailfish | |
|---|---|---|---|---|---|
| | Mazatlán | Buena Vista | San Diego | Mazatlán | Buena Vista |
| Number of stomachs | 143 | 303 | 248 | 151 | 14 |
| Invertebrates: | | | | | |
| Crustacea: | | | | | |
| *Pasiphaea affinis* | --- | T | --- | --- | -- |
| Unidentified shrimp | 1 | --- | --- | 1 | -- |
| Unidentified galatheid | --- | T | --- | --- | -- |
| *Portunus xantusii* | --- | --- | --- | 2 | -- |
| Cephalopoda: | | | | | |
| *Japetella* sp. | --- | X | --- | --- | -- |
| *Argonauta* sp. | 17 | X | T | 26 | 7 |
| Squid | 54 | 69 | 4 | 38 | 50 |
| Fishes: | | | | | |
| Lamnidae: | | | | | |
| *Alopias vulpinus* | --- | --- | 1 | --- | -- |
| Clupeidae: | | | | | |
| *Etrumeus teres* | --- | 27 | --- | --- | 14 |
| *Sardinops sagax* | --- | --- | 2 | --- | -- |
| Engraulidae: | | | | | |
| *Engraulis mordax* | --- | --- | 90 | --- | -- |
| Synodontidae: | | | | | |
| *Synodus scituliceps* | X | --- | --- | 2 | -- |
| Myctophidae: | | | | | |
| Unidentified | --- | T | --- | --- | -- |
| Gonostomatidae: | | | | | |
| *Vinciguerria lucetia* | --- | --- | --- | --- | 7 |
| Congridae: | | | | | |
| *Ariosoma balearica* | --- | --- | --- | --- | X |
| Scomberesocidae: | | | | | |
| *Cololabis saira* | --- | --- | 15 | --- | -- |
| Belonidae: | | | | | |
| Unidentified [1] | 4 | --- | --- | --- | -- |
| Exocoetidae: | | | | | |
| Unidentified | 1 | 1 | 2 | --- | -- |
| Fistulariidae: | | | | | |
| *Fistularia* sp.[2] | 31 | 11 | --- | 40 | 14 |
| Serranidae: | | | | | |
| *Diplectrum pacificum* | --- | --- | --- | 1 | -- |
| Lobotidae: | | | | | |
| *Lobotes pacificus* | 2 | --- | --- | --- | -- |
| Lutjanidae: | | | | | |
| *Lutjanus peru* | --- | X | --- | --- | -- |
| Carangidae: | | | | | |
| *Caranx caballus* | --- | 2 | --- | --- | -- |
| *C. hippos* | --- | --- | --- | 1 | -- |
| *Chloroscombrus orqueta* | --- | --- | --- | 1 | -- |
| *Decapterus hypodus* | 1 | 6 | --- | 1 | -- |
| *Hemicaranx* sp. | 1 | --- | --- | 6 | -- |
| *Naucrates ductor* | 4 | 1 | --- | 5 | 14 |
| *Oligoplites refulgens* | X | --- | --- | --- | -- |
| *Selar crumenopthalmus* | 2 | --- | --- | 1 | -- |
| *Trachurus symmetricus* | --- | --- | 31 | --- | -- |
| *Vomer declivifrons* | 2 | --- | --- | 4 | -- |
| Unidentified | --- | --- | --- | 8 | -- |
| Pomadasyidae: | | | | | |
| *Lythrulon flaviguttatum* | --- | T | --- | --- | -- |
| Scorpidae: | | | | | |
| *Medialuna californiensis* | --- | --- | 2 | --- | -- |
| Chaetodontidae: | | | | | |
| *Chaetodon humeralis* | 4 | X | --- | 6 | -- |
| Trichiuridae: | | | | | |
| *Trichiurus nitens* | --- | T | --- | --- | -- |
| Scombridae: | | | | | |
| *Auxis thazard* | 3 | 3 | --- | 2 | -- |
| *Scomber japonicus* | 2 | 7 | --- | 15 | -- |
| *Thunnus* sp. | 2 | --- | --- | --- | -- |
| *Sarda chiliensis* | --- | --- | 1 | --- | -- |
| *Scomberomorus* sp. | X | --- | --- | --- | -- |
| Unidentified | --- | 1 | --- | --- | 7 |
| Scorpaenidae: | | | | | |
| *Scorpaena sonorae* | --- | --- | --- | 1 | -- |
| Stromateidae: | | | | | |
| *Cubiceps* sp. | --- | X | --- | --- | X |
| *Palometa* sp. | --- | --- | --- | 1 | -- |
| Mugilidae: | | | | | |
| *Mugil cephalus* | --- | --- | T | --- | -- |
| Unidentified [3] | 5 | --- | --- | 5 | -- |
| Polynemidae: | | | | | |
| *Polydactylus* sp.[4] | 6 | --- | --- | 34 | -- |

| | | | | | |
|---|---|---|---|---|---|
| Balistidae: | | | | | |
| *Balistes* sp.[5] | 8 | X | --- | 5 | X |
| *Alutera scripta* | 4 | --- | --- | --- | -- |
| Ostraciidae: | | | | | |
| *Lactoria diaphana* | 1 | --- | --- | --- | -- |
| Tetraodontidae: | | | | | |
| *Sphoeroides* sp.[6] | 6 | X | --- | 15 | -- |
| *Lagocephalus lagocephalus* | --- | X | --- | --- | X |
| Unidentified fish remains | 27 | 22 | 6 | 52 | 14 |

[1] Some probably *Strongylura* sp.
[2] Several identified as *F. corneta.*
[3] Some identified as *Mugil curema.*
[4] Many were identified as *P. opercularis.*
[5] Some identified as *B. polylepis.*
[6] Some identified as *S. lobatus.*

By volume (table 2), squid ranked first for Mazatlán and Buena Vista samples (63% and 49%, respectively). Round herring ranked second (30%) for the Buena Vista samples, but at Mazatlán no single group was of major secondary importance. Anchovies were most important (60%) and Jack mackerel ranked second (27%) at San Diego.

By number of individual food items (Table 3), the paper nautilus ranked first (36%) for the Mazatlán fish, though it composed only 7% by volume. Otherwise, the ranking of food categories is quite similar to that made on the basis of volume.

The diversity of fishes utilized as food by striped marlin was greatest at Mazatlán, with a total of 22 species found in the diet. Buena Vista striped marlin fed on 17 species, and San Diego marlin utilized only 9 species.

TABLE 2.—*Percent of volume for major food categories (those 5% or more) for marlin and sailfish in each area*

[Data for 1967 and 1968 combined except the San Diego figures are based on 1968 data only]

| | Striped marlin | | | Sailfish | |
|---|---|---|---|---|---|
| | Mazatlán | Buena Vista | San Diego | Mazatlán | Buena Vista |
| *Argonauta* sp. | 7 | --- | --- | 10 | -- |
| Squid | 63 | 49 | --- | 21 | 35 |
| *Etrumeus teres* | --- | 30 | --- | --- | 29 |
| *Engraulis mordax* | --- | --- | 60 | --- | -- |
| *Cololabis saira* | --- | --- | 5 | --- | -- |
| *Fistularia* sp. | 5 | --- | --- | 8 | 22 |
| *Naucrates ductor* | --- | --- | --- | --- | 7 |
| *Trachurus symmetricus* | --- | --- | 27 | --- | -- |
| *Scomber japonicus* | --- | 7 | --- | 5 | -- |
| *Mugil* sp. | --- | --- | --- | 5 | -- |
| *Polydactylus* sp. | --- | --- | --- | 27 | -- |
| *Balistes* sp. | 7 | --- | --- | --- | -- |
| Unidentified fish remains | --- | --- | --- | 14 | -- |

TABLE 3.— *Percent of number of individuals for major food categories (those 5% or more) for marlin and sailfish in each area, 1967 and 1968 combined*

| | Striped marlin | | | Sailfish | |
|---|---|---|---|---|---|
| | Mazatlán | Buena Vista | San Diego | Mazatlán | Buena Vista |
| *Argonauta* sp. | 36 | --- | --- | 25 | -- |
| Squid | 34 | 57 | --- | 9 | 47 |
| *Etrumeus teres* | --- | 19 | --- | --- | 11 |
| *Engraulis mordax* | --- | --- | 92 | --- | -- |
| *Vinciguerria lucetia* | --- | --- | --- | --- | 16 |
| *Fistularia* sp. | 13 | 6 | --- | 8 | 18 |
| *Scomber japonicus* | --- | 12 | --- | --- | -- |
| *Polydactylus* sp. | 5 | --- | --- | 42 | -- |

### Seasonal differences

Since information on the fluctuations in abundance of prey species in the environment is not available, we simply report fluctuations in the stomach contents. The 1967 data were insufficient to reveal seasonal trends, so only the 1968 food volume data are presented. At Buena Vista (fig. 1) during the first three days of sampling (April 18-20) squid were by far the major item in the stomach contents. A few days later the average volume of squid per stomach declined while the volume of round herring increased. During the first two-thirds of May, squid increased while the volume of round herring decreased. Round herring increased again in the diet in early June, after which both round herring and squid declined. Other species were less prevalent in the diet. The volume of chub mackerel increased substantially in late May. Frigate mackerel (*Auxis thazard*) and green jack (*Caranx caballus*) increased in late June at a time when squid and round herring volumes were low, so that frigate mackerel, squid, and green jack were of similar importance. Mexican scad first appeared in striped marlin stomachs at Buena Vista in early June and by mid-July were more important than squid, although all volumes were very low at this time. The stomach samples obtained in November 1969 from Buena Vista revealed two species previously found only in the Mazatlán material—triggerfish and a butterflyfish (*Chaetodon humeralis*).

At Mazatlán the average volume of squid in striped marlin stomachs sampled increased threefold from mid-April to late April and early May, after which it decreased sharply (fig. 2). Other foods were of minor importance, and none showed a compensatory increase when the volume of squid declined. The Mexican scad occurred in stomachs sampled in December 1968, whereas previously it was found only in Buena Vista striped marlin.

At San Diego anchovies in the diet increased from an average of 8.7 oz per stomach in early September to 9.9 oz in mid-September and then declined steadily (fig. 3). The volume of jack mackerel, the second most important food species, was

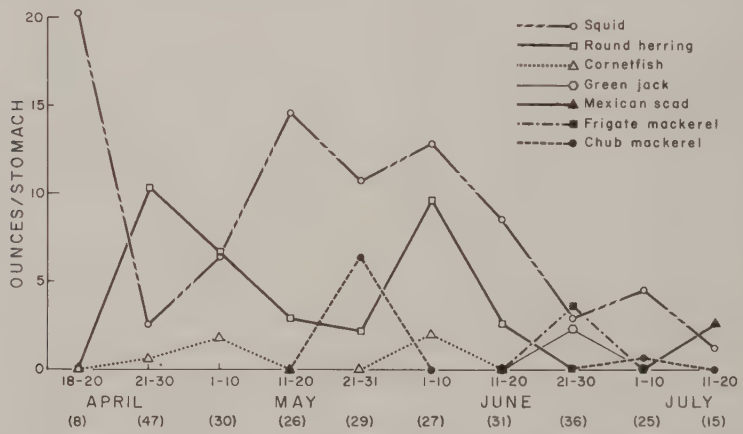


FIGURE 1.—Temporal variations in average volume of the major prey species in striped marlin stomachs sampled in 1968 at Buena Vista. Data are expressed as fluid ounces per stomach containing food for each species which comprised at least 10% of the volume during one or more 10-day sample periods. Amounts less than 0.5 oz are not shown. Sample sizes are given in parentheses below dates.

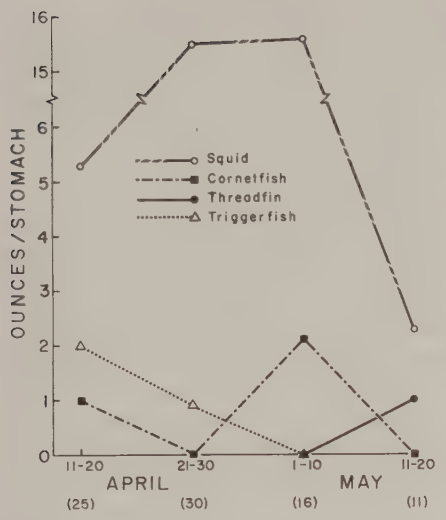


FIGURE 2.—Temporal variations in average volume of the major prey species in striped marlin stomachs sampled in 1968 at Mazatlán. Data are expressed as fluid ounces per stomach containing food for each species which comprised at least 10% of the volume during one or more 10-day sample periods. Amounts less than 0.5 oz are not shown. Sample sizes are given in parentheses below dates.

lowest in late September and greatest in mid-October, when it exceeded anchovies in volume per stomach. Sauries were a minor component in the diet, but in mid-October the volume increased to 3.4 oz per stomach.

### *Yearly differences*

There were some striking differences between the 1967 and 1968 data for Mazatlán marlin (not shown separately in tables 1-3). In 1967 the paper nautilus was the major food item (40% by volume), and squid and mullets each constituted 21% by volume. In 1968 the paper nautilus constituted only 0.1%, while squid constituted 75% and mullets were absent. At Buena Vista and San Diego yearly differences were less pronounced. In 1967 the major food items at Buena Vista were round herring, 41% by volume; squid 36%; and chub mackerel 16%. For the same dates in 1968 squid constituted 55%, round herring 18%, and chub mackerel 20%. Anchovies constituted 94% by number of the diet of San Diego marlin in 1967 and 91% in 1968.

The overall average volume of food per stomach containing some food was similar at Mazatlán, Buena Vista, and San Diego during 1968 (14.3 to 14.9 oz per stomach). In 1967, at San Diego the average volume was much higher (22.9 oz. per stomach). Bias in technique was a factor in this difference; only total volumes were measured in 1967, and they included stomach juices and unidentified debris which were excluded in 1968, when volume of each prey species was measured individually.

## SAILFISH

### *Locality differences*

The data from the two localities are scarcely comparable, since only 11 stom-

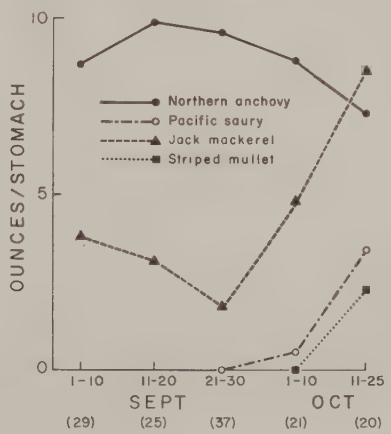


FIGURE 3.—Temporal variations in average volume of the major prey species in striped marlin stomachs sampled in 1968 at San Diego. Data are expressed as fluid ounces per stomach containing food for each species which composed at least 10% of the volume during one or more 10-day sample periods. Amounts less than 0.5 oz are not shown. Sample sizes are given in parentheses below dates.

achs with food were found at Buena Vista during the time the sailfish were being caught at Mazatlán. The major foods by volume, however, were threadfin 27%, squid 21%, and paper nautilus 10% at Mazatlán, and squid 35%, round herring 29%, and cornetfish 22% at Buena Vista.

### *Seasonal and yearly differences*

In the 1968 studies at Mazatlán (fig. 4), cornetfish predominated in average volume per stomach during mid-April, squid in late April and early May, and threadfins through the rest of May. In early June the paper nautilus composed the major portion of the stomach content volume. Data from 1967 and from Buena Vista in 1968 were insufficient to reveal seasonal trends in utilization of prey species.

For the same calendar dates at Mazatlán, 44% of the diet by volume in 1967 consisted of squid, which constituted 59% in 1968. Threadfins constituted 17% in 1967, but only 0.6% in 1968, and triggerfish constituted 6% in 1967, but were not found during the same period in 1968.

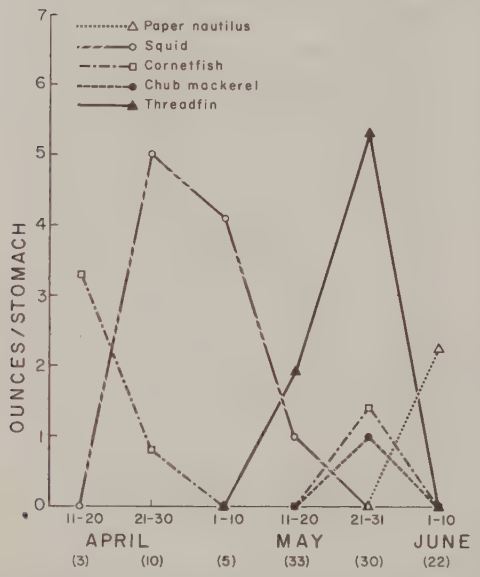


FIGURE 4.—Temporal variations in average volume of the major prey species in sailfish stomachs sampled in 1968 at Mazatlán. Data are expressed as fluid ounces per stomach containing food for each species which composed at least 10% of the volume during one or more 10-day sample periods. Amounts less than 0.5 oz are not shown. Sample sizes are given in parentheses below dates.

### *Comparison with striped marlin diet*

During the period from mid-April to mid-May when both striped marlin and sailfish were being landed at Mazatlán, there was much similarity in the diets of the two species (figs. 2 and 4). In the diets of both species, squid volume went through a cycle of increase and decline, but squid dominated the diets from late April to early May. In mid-May threadfin became important in the diets of both species, though it was the dominant food group only for the sailfish. Cornetfish formed the second most important group in both diets but constituted only 5% of the striped marlin diet and 10% of the sailfish diet. Both striped marlin and sailfish utilized squid and paper nautilus plus 11 species of fishes during this time, but only five of the 11 species occurred in both diets. Triggerfish constituted 7% of the marlin diet but were not utilized at all by sailfish during the same period.

The overall average total volume of food per stomach for sailfish at Mazatlán was 6.9 oz, approximately half that found for striped marlin.

## DISCUSSION

Because of the scarcity of information on relative abundance of prey species, it is difficult to assess whether the billfish studied have preference for certain foods. Some information is available for the San Diego area from the California Cooperative Fisheries Investigations (CalCOFI) pelagic fish survey cruises and Bureau of Commercial Fisheries egg and larva surveys, but comparable data are lacking for the Mexican study areas. We are thus limited to consideration of striped marlin

at San Diego. Generally the CalCOFI studies have shown that the anchovy population increased ninefold between 1951 and 1968 (Ahlstrom, 1968) and that the saury population has remained relatively constant during the last 20 years (Smith, Ahlstrom, and Casey, in press). Hubbs and Wisner (1953) found that the major foods of 32 striped marlin taken in the sport fishery at San Diego in 1951 were Pacific sauries 72% by volume, northern anchovies 12%, Pacific sardines (*Sardinops sagax*) 9%, and jack mackerel 3%. They found that anchovies predominated in the diet in 1952 (Wisner, personal communication). It may therefore be that the importance of anchovies as a striped marlin food off southern California existed prior to the apparent increase in anchovy abundance.

The dominance of fish and virtual absence of squid from the diet of striped marlin at San Diego is in sharp contrast to the composition of the diet at the Mexican areas where squid were dominant. The CalCOFI data include observations of squid but do not give an adequate indication of their abundance relative to the abundance of pelagic fishes. These reports do indicate that squid (primarily *Loligo opalescens*) occur concurrently with marlin off San Diego (California Marine Research Committee, 1966a, 1966b, 1969, and unpublished data).

The diet of striped marlin in other areas of the Pacific also varies with locality and time, generally with either squids, sauries, anchovies, scombrids, or carangids as the major food in a given study. Off New Zealand, Morrow (1952) reported saury (*Scomberesox saurus*) as the major food species, whereas Baker (1966) found squids and carangids to be the major foods. LaMonte (1955) reported only squid in the stomachs of striped marlin off Peru and Chile, but de Sylva (1962) noted anchovy (*Engraulis ringens*), squid, jack mackerel (*Trachurus symmetricus murphyi*), and saury (*Scomberesox equirostrum*) in the stomachs of three striped marlin taken off Chile. Royce (1957) found "tunalike fishes, some identifiable as *Auxis*, and miscellaneous remains of other fish, shrimp, and squid" in stomachs of striped marlin from equatorial waters of the central Pacific.

Other authors report food habits of sailfish from other areas to be similar to our findings off Mexico. In nine sailfish stomachs from the central Pacific, Royce (1957) noted that squid was the predominant food, with octopus, nautilus, *Alepisaurus*, one bramid, and one pilot fish also occurring. Voss (1953) reported fishes (principally scombrids, carangids, hemirhamphids, and belonids) to be the most important foods (83% by number) of 241 sailfish off Florida, with cephalopods, the majority of which were paper nautilus, composing the remainder of the diet.

Most of the prey species identified in our study are epipelagic, but several are typically inshore species such as the butterflyfish, the sea bass (*Diplectrum pacificum*), the tripletail (*Lobotes pacificus*), the scorpionfish (*Scorpaena sonorae*), the triggerfish, and the cornetfish. Although inshore species sometimes occur offshore in association with floating objects (Hunter and Mitchell, 1967; Hunter, 1968), the predominance of the occurrences of these species at Mazatlán, where an extensive shelf occurs in the fishing area, suggests that at least some may have been captured in their normal habitat. Also, the lack of deep water off Mazatlán may explain the lower utilization of pelagic fishes by billfish there as compared with Buena Vista and San Diego. At Buena Vista several intermediate to deep-water forms occurred: the octopod *Japetella* sp., the shrimp *Pasiphaea affinis*, a myctophid, a gonostomatid, and a trichiurid.

We did not have an opportunity to observe feeding behavior, but we found two carangids in the stomach contents of

striped marlin which appeared to have been impaled by the bill prior to ingestion.

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# GENIC POLYMORPHISM OF BLOOD PROTEINS FROM WHITE MARLIN

By Philip H. Edmunds

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## ABSTRACT

Over 100 white marlin (*Tetrapturus albidus*) were analyzed for each of seven proteins to discover genetically controlled variations that may be useful in subpopulation studies. The iron-binding plasma protein, transferrin, and the enzymes esterase, 6-phosphogluconate dehydrogenase (PGD), and malate dehydrogenase (MDH) each occur in multiple electrophoretically distinct forms: two of transferrin, two of esterase, five of PGD, and three of MDH. Transferrin, esterase, PGD, and MDH patterns differ from one marlin to another. Individual differences apparently reflect genic polymorphism: two codominant alleles at one autosomal locus controlling three transferrin patterns, two codominant alleles at another autosomal locus controlling three esterase patterns, three codominant alleles at a third autosomal locus controlling four PGD patterns, and two codominant alleles at a fourth locus controlling two MDH patterns. Hemoglobin and the enzymes lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PD) also occur in multiple electrophoretically distinct forms: six of hemoglobin, four of LDH, and one or two of G6PD. None of the three proteins exhibit genic variations. Geographical distributions of transferrin, esterase, PGD, and MDH variants as yet provide no reason to assign marlin from four widely separated areas in the western North Atlantic to different subpopulations. This fact and information about migrations and spawning activity suggest there are at least two subpopulations of white marlin: one in the western North Atlantic and one or more in the South Atlantic.

# GENIC POLYMORPHISM OF BLOOD PROTEINS FROM WHITE MARLIN

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Recognizing and defining independent self-sustaining breeding populations is of fundamental importance to fishery management. These units usually occupy different geographical areas, as with albacore (Suzuki, Morio, and Mimoto, 1959), skipjack (Fujino and Kang, 1968a), and sockeye salmon (Hodgins, Ames, and Utter, 1969). They may have different growth rates, as with sardines (Vrooman, 1964) and hake (Utter and Hodgins, 1969). They may also differ with respect to meristic characters, as with plaice (Purdom and Wyatt, 1969) and herring (Ridgway, Lewis, and Sherburne, 1970) or with respect to morphological features, as with cod (Møller, 1966). Marr (1957) defined them as subpopulations.

Subpopulations are unlikely to exist if individual fish move freely throughout the species' geographical range or if all individuals spawn at the same time in the same place. The existence of subpopulations is plausible for species whose members exhibit limited movement or split spawnings. Migratory behavior and the time and location of spawning are therefore important considerations in population analyses.

When my associates and I began analyses of white marlin (*Tetrapturus albidus*) populations in 1967, there was scant information on any of these three factors. De Sylva and Davis (1963) stated that some of the female white marlin examined in the spring at Cat Cay, Bahamas, were approaching spawning condition, whereas all females examined during the summer at Ocean City, Md., were spent. They assumed that the population of marlin off Ocean City during summer was the same population present during spring in the Florida Straits. Evidence from tagging studies (Mather, 1967) supports this assumption. De Sylva and Davis (1963) concluded that spawning occurs somewhere between the Bahamas and the Middle Atlantic Bight. Occurrence of 10- to 12-cm postlarval white marlin in this area (de Sylva, 1963) is consistent with their conclusion. Mather (1967) reported that four white marlin tagged during summer in the Middle Atlantic Bight were recaptured during winter off Venezuela and the Guianas, and another tagged fish moved from the Florida Straits to the western Gulf of Mexico.

We began studies to ascertain whether

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NOTE.—The Narragansett Marine Game Fish Research Laboratory was transferred in October 1970 from the Bureau of Sport Fisheries and Wildlife, U.S. Fish and Wildlife Service, Department of the Interior, to the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Department of Commerce.

as with cod (Møller, 1966). Marr (1957) white marlin subpopulations exist. These studies involve electrophoretic analyses of selected proteins from white marlin blood and tissues. They comprise three phases: first, surveying large numbers of individuals to detect genetically controlled variations among them; then, classifying individuals according to particular genetic types; and finally, comparing lots of fish, separated in space or time, on the basis of the classifications. The underlying principle is that fish with a particular genetic type can be significantly more common in one lot than in another only if the two lots are from different subpopulations.

So far we have identified four genetically variable systems, each with at least three genetic types. The main purpose of this report is to describe these systems. I also try to relate these biochemical data to other information on the relationship of white marlin populations from widely separated geographical areas.

For help in getting white marlin blood and tissue samples I am indebted to R. F. McCann and his staff at the Bimini Big Game Fishing Club, to Charles Law, weighmaster at the Atlantic City State marina, to Ruben Jaen C. of the Playa Grande Yachting Club near La Guaira, Venezuela, and to Joe Yurt and the members of the New Orleans Big Game Fishing Club, especially Herman Prager, Jr., Harley B. Howcott, W. Kohlmann Reed, Lloyd Morgau, and Edmund Hawkins. Alan R. Bostock and James I. Sammons III collected much of the sample material and assisted with analyses in the laboratory. I am also indebted to Connie R. Arnold, John G. Casey, John R. Clark, Anthony Pacheco, George J. Ridgway, and Lewis T. Smith for advice on preliminary drafts of the manuscript.

## MATERIALS AND METHODS

Most of the white marlin sampled were caught during organized sportfishing tournaments at Atlantic City, N.J., in early August, 1967, 1968, and 1969; at Ocean City, Md., in early September 1968; at Bimini, Bahamas, in early April, 1968 and 1969; at South Pass, La., from early July to early September 1968; and during early July 1969; and at Playa Grande, Venezuela, in mid-September, 1968 and 1969. Participating boats normally range up to 50 miles from these ports during the competitions. Three nontournament marlin were caught in early June 1968 during longline fishing aboard the research vessel DOLPHIN along the western edge of the Gulf Stream northeast of Cape Hatteras, N.C. Four others were caught on hook and line southwest of Martha's Vineyard, one in mid-July and three in mid-August 1968.

Blood specimens were collected from the thin-walled sinus directly behind the ventricle of the heart into Vacutainer tubes containing 12 mg lithium oxalate anticoagulant. There was little or no clotting in the sinus even in marlin exposed to air and sun for several hours before reaching the dock. We kept whole blood iced or refrigerated in the field, then separated plasma from cells by centrifugation after returning to the laboratory. Analyses of red blood cells (RBC) were carried out as soon thereafter as possible—usually within 1 week—to minimize effects of spoilage, but plasma samples were stored at or below —7°C until it was convenient to analyze them. We stored 5- to 10-gram pieces of heart, liver, gonad, and skeletal muscle in plastic bags, using a single bag for all tissues from each fish. Tissues were iced, refrigerated, or frozen in the field, depending on available facilities, and stored at or below —7°C in the laboratory.

We prepared RBC hemolysates by mixing one volume of washed packed RBC with approximately three volumes of distilled water. This mixture was agitated

thoroughly with a glass stirring rod before and after addition of two drops of 0.2 M sucrose. Sucrose increased solution density, making it easier to pipette samples into the electrophoretic cell. The mixture was centrifuged for 2 to 4 minutes at 1000 *g* to get the cherry-red supernatant solution used for analyses of hemoglobin and RBC enzymes.

Fragments of heart and gonad were homogenized with twice their weight of 0.2 M sucrose. Slightly greater amounts of sucrose solution were needed for homogenization of skeletal muscle and liver. We centrifuged these slurries for 30 minutes at 2700 *g* and stored supernatant solutions in stoppered glass vials at or below —7°C until it was convenient to analyze them.

We carried out electrophoretic separations of plasmas, hemoglobin solutions, and tissue homogenates in slabs of polyacrylamide gel. Gels were prepared at different concentrations (w/v) from a 19:1 mixture (w/w) of acrylamide and N,N′-methylenebisacrylamide that was dissolved in a buffer solution containing 0.1 ml N,N,N′,N′-tetramethylethylenediame/100 ml buffer. Addition of ammonium persulfate to a final concentration of 0.8 mg/ml caused the solution to polymerize in 30 to 45 minutes. Circulation of ethylene glycol at a temperature of 7°C through the electrophoretic cell prevented overheating of the gels during electrophoresis. Following electrophoresis we used histochemical staining procedures to identify selected proteins on the gels. Technical details related to gel concentration, buffer composition, sample size, voltage during electrophoresis, and staining procedures differ from one protein to another and therefore are summarized separately below for each protein.

Histochemical staining procedures are specific for certain proteins. They produce narrow bands of color on the transparent gels wherever such proteins are located and only at these locations. It is sometimes convenient to take advantage of this fact by using the term "bands" in place of the more cumbersome phrase "electrophoretically distinct proteins," and I do so here.

### *Esterase (Es)*

Gel concentration: 6%. Buffer: a continuous system described by Peacock, Bunting, and Queen (1965), using tris (hydroxymethyl) aminomethane / EDTA / boric acid, pH 8.4, in gels and electrode compartments (used for plasma); a continuous system, pH 7.5, composed of $1.9\times10^{-2}$ M tris and $5\times10^{-3}$ M citric acid in gels and electrode compartments (for RBC hemolysates and tissue homogenates); plasma esterase stained much more intensely after separation in tris/citric acid than in tris/EDTA/boric acid. Sample size: 5 or 10 $\mu$l of plasma; 10 or 20 $\mu$l of RBC hemolysates or tissue homogenates. Voltage during electrophoresis: 20-minute prerun at 350 v before loading of samples, then 350 v for approximately 2½ hours. Staining: diazonium method as described by Markert and Hunter (1959) except for the following modifications: substitution of pH 7.4, 0.2 M tris/HCl buffer for pH 6, 0.2 M phosphate buffer; substitution of $\alpha$-naphthyl acetate for $\alpha$-naphthyl butyrate as substrate; reduction of Fast Blue RR salt concentration from 1.0 mg/ml to 0.5 mg/ml.

### *Transferrin (Tf)*

Gel concentration: 6%. Buffer: a discontinuous system described by Davis (1964), using tris/HCl, pH 8.9 and pH 6.7, in gels and tris/glycine, pH 8.3, in electrode compartments. Sample size: 50 $\mu$l, including 45 $\mu$l of plasma and 5 $\mu$l of $9\times10^{-4}$ M ferric chloride solution; we added iron (i.e., ferric iron) to plas-

ma samples before electrophoresis since Baker (1967) reported that this procedure enhanced staining of avian transferrin. Voltage during electrophoresis: 200 v until samples "stack" in spacer gel, then 350 v for about 2¾ hours. Staining: sodium acetate/2,4-dinitroso-1,3-naphthalenediol/hydroquinone.

#### *6-phosphogluconate dehydrogenase (PGD)*

Gel concentration: 6%. Buffer: the continuous tris/EDTA/boric acid system used for plasma esterase. Sample size: 20 μl of RBC hemolysates and tissue homogenates. Voltage during electrophoresis: same as for plasma esterase. Staining: tetrazolium method as described by Bender and Ohno (1968) except that we reduced the amounts of components in 10 ml of staining solution as follows: 6-phosphogluconate disodium salt from 10 mg to 2 mg, phenazine methosulfate from 2 mg to 1.6 mg, MTT tetrazolium from 2 mg to 1.6 mg, and NADP from 2 mg to 1.3 mg.

#### *Malate dehydrogenase (MDH)*

Gel concentration: 4% for tissue homogenates; 6% for RBC hemolysates. Buffer: a discontinuous system described by Poulik (1957), using tris/citric acid, pH 8.6, in gels and sodium hydroxide/boric acid, pH 8.0, in electrode compartments; we diluted the electrode buffer 1:10 with distilled water; in our hands the Poulik buffers gave better results than the tris/glycine system used for lactate dehydrogenase (LDH) and the tris/EDTA/boric acid system used for esterase and PGD. Sample size: 10 μl of tissue homogenates; 20 μl of RBC hemolysates. Voltage during electrophoresis: 350 v for 2 to 2½ hours. Staining: tetrazolium method as described by Goldberg (1963) except that we reduced final concentrations of some components in the staining solution as follows: NBT, from 40 mg to 15 mg/ml; NAD, from 15 mg to 12 mg/ml.

#### *Lactate dehydrogenase (LDH)*

Gel concentration: 4%. Buffer: a discontinuous system with gels made up in pH 8.9, $3.79\times10^{-1}$ M tris/HCl and electrode compartments filled with a pH 8.3 mixture containing $9.9\times10^{-3}$ M tris and $7.7\times10^{-2}$ M glycine. Sample size: 10 μl of tissue homogenates; 20 μl of plasma or RBC hemolysates. Voltage during electrophoresis: 350 v for about 2¾ hours. Staining: tetrazolium method described by Goldberg (1963) except that we reduced final concentrations of NBT and NAD in the staining solution as for malate dehydrogenase.

#### *Glucose-6-phosphate dehydrogenase (G6PD)*

Gel concentration: 4% and 6%. Buffer: the discontinuous tris/HCl/glycine system used for LDH (tissue homogenates and RBC hemolysates); the continuous tris/EDTA/boric acid system used for plasma esterase (only RBC hemolysates). Sample size: 10 and 20 μl of tissue homogenate or RBC hemolysates. Voltage during electrophoresis: 350 v for 2½ to 2¾ hours. Staining: tetrazolium method as described by Nakano and Whiteley (1965) except that we used pH 8.3, $1.4\times10^{-2}$ M tris/HCl buffer in place of pH 8.6, 1 M tris/HCl buffer.

#### *Hemoglobin (Hb)*

Gel concentration: 6%. Buffer: the discontinuous tris/HCl/glycine system used for LDH and G6PD. Sample size: 10 or 20 of RBC hemolysates. Voltage during electrophoresis: 350 v for about 1¾ hours. Staining: none required.

## DESCRIPTION AND INTERPRETATION OF ELECTROPHORETIC PATTERNS

### *Esterase*

This enzyme occurs in white marlin blood plasma in two major electrophoretically distinct forms. The one with greater electrophoretic mobility is here designated Es–1A and the other Es–1B. Each has associated with it a minor band that is visible on some gels but absent from others.

Some fish have only Es–1A, some have only Es–1B, and some have both. In other words, most white marlin exhibit one of three plasma esterase phenotypes (fig. 1, a). Plasma esterase activity was undetectable in 3 of 351 marlin tested.

I believe these individual differences result from segregation of two codominant autosomal alleles, here designated $Es\text{–}1^A$ and $Es\text{–}1^B$. According to this interpretation, fish with only band Es–1A are homozygotes with genotype $Es\text{–}1^A/Es\text{–}1^A$; fish with only band Es–1B are homozygotes with genotype $Es\text{–}1^B/Es\text{–}1^B$; fish with both bands are heterozygotes with genotype $Es\text{–}1^A/Es\text{–}1^B$.

This interpretation is based on four kinds of evidence. First, the variations are similar to those in deer mice (Randerson, 1965), house mice (Popp, 1966), and chickens (Grunder, 1968). Breeding experiments prove that esterase variations are controlled by codominant autosomal alleles in these species. Second, our population data conform to the Hardy-Weinberg distribution, which is an algebraic model commonly used for analyses of hereditary traits in populations (Srb and Owen, 1958). There is good agreement between theoretical frequencies of the three phenotypes based on this mode of inheritance and frequencies actually observed in different geographical areas (table 1). Third, esterase patterns are unrelated to sex or size of the fish (table 2). Finally there is no apparent relation between esterase variations and the reproductive cycle; I found all phenotypes among marlin with ripening ovaries and testes as well as among those with inactive gonads.

Tissue esterases are more complex than the plasma enzyme. Separations of heart, liver, skeletal muscle, ovary, and testis homogenates are similar to each other.

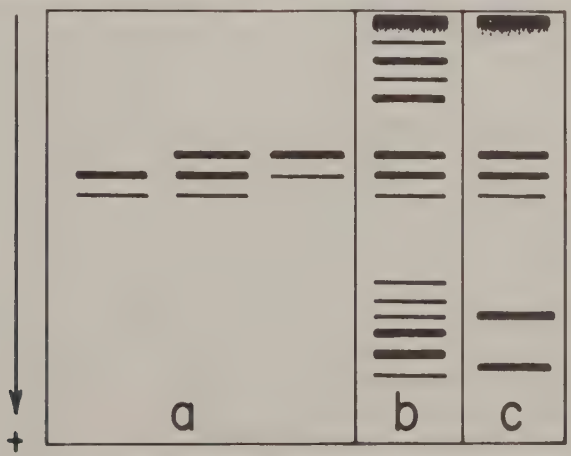


FIGURE 1.—Electrophoretic patterns of esterase from various tissues: *a*. Blood plasma showing, from left to right, phenotypes Es-1A, Es-1AB, and Es-1B; *b*. Heart muscle (skeletal muscle, liver, ovary, and testis are similar to heart)—each Es-1 phenotype was observed in one fish or another, but only Es-1AB is illustrated; *c*. RBC.

TABLE 1.—*Incidence of Es-1 variants in different geographical areas, with comparisons between observed distributions of phenotypes and (in parentheses) theoretical distributions based on inheritance controlled by two codominant alleles*

| Area | Number of fish | Phenotype | | | p[1] | $\chi^2$ | Probability df=1 |
|---|---|---|---|---|---|---|---|
| | | Es-1A | Es-1AB | Es-1B | | | |
| Middle Atlantic Bight | 151 | 32 (34.7) | 81 (75.4) | 38 (40.8) | 0.480 | 0.82 | 0.50>P>0.25 |
| Florida Straits | 96 | 21 (22.5) | 51 (47.9) | 24 (25.5) | 0.484 | 0.39 | 0.75>P>0.50 |
| Northern Gulf of Mexico | 69 | 16 (16.2) | 35 (35.1) | 18 (18.3) | 0.485 | 0.01 | 0.95>P>0.90 |
| Lower Caribbean Sea | 32 | 5 ( 5.2) | 16 (15.4) | 11 (11.3) | 0.406 | 0.04 | 0.90>P>0.75 |
| Total | 348 | 74 (78.3) | 183 (173.3) | 91 (95.7) | 0.475 | 1.01 | 0.50>P>0.25 |

[1] Frequency of allele *Es-1*$^{A}$ estimated from phenotypic proportions.

TABLE 2.—*Contingency test for independence of Es-1 phenotypes and sex and size of fish*

| | Frequency of phenotypes[1] | | | $\chi^2$ | df | Probability |
|---|---|---|---|---|---|---|
| | Es-1A | Es-1AB | Es-1B | | | |
| Sex: | | | | | | |
| Male | 24(23.0) | 64(61.1) | 28(31.1) | 1.08 | 2 | 0.75>P>0.50 |
| Female | 31(32.0) | 82(84.8) | 48(44.2) | | | |
| Fork length (cm): [2] | | | | | | |
| ≤ 150.0 | 5( 6.2) | 21(16.7) | 5( 8.0) | | | |
| 150.1-160.0 | 26(25.2) | 63(67.4) | 36(32.4) | 3.41 | 6 | 0.90>P>0.75 |
| 160.1-170.0 | 19(18.1) | 49(48.5) | 22(23.3) | | | |
| ≥ 170.1 | 9( 9.5) | 25(25.3) | 13(12.2) | | | |

[1] Number of fish with each phenotype actually observed and (in parentheses) expected number, calculated from row and column totals.
[2] Here defined as distance from tip of lower jaw to fork of caudal fin.

All contain Es–1 and two other zones of activity that are absent from plasma preparations (fig. 1, b). One zone about halfway between Es–1 and the origin contains up to four bands that sometimes appear smudged. A second zone more anodal than Es–1 contains two prominent and three or four variably faint bands. RBC hemolysates have Es–1 and a more anodal zone with two well-separated prominent bands (fig. 1, c). All tissues and some plasmas exhibit esterase activity at the origin.

There are considerable differences among individual marlin with respect to tissue esterases. Es–1 variations are evident in all tissues. But I can ascertain Es–1 phenotypes only in skeletal muscle and RBC material because the bands in heart, liver, and gonad preparations are diffuse. Staining intensity of enzymes from the other major zones varies. Bands that are well stained in preparations from one fish are virtually undetectable in others. This gradation of staining intensity makes the distinction between a band that is merely poorly stained and one that is absent highly subjective. Unfortunately this distinction must be made in developing hypotheses about genetic control of variations. I foresee no practical solution to this problem and therefore feel that identification of other genetically controlled variation of tissue esterases is unlikely.

### *Transferrin*

This iron-binding protein of blood plasma occurs in white marlin in two major electrophoretically distinct forms.

The band with greater electrophoretic mobility is here designated Tf–A and the other Tf–B. Each has associated with it a minor band that is visible on some gels and absent from others.

Some white marlin have only Tf–A, some have only Tf–B, and some have both. Most white marlin thus exhibit one of three transferrin phenotypes (fig. 2). We failed to detect transferrin in only 1 of 350 fish tested.

I believe that these individual differences result from segregation of two codominant autosomal alleles, here designated $Tf^A$ and $Tf^B$. According to this interpretation, fish with only band A are homozygotes with genotype $Tf^A/Tf^A$; fish with only band B are homozygotes with genotype $Tf^B/Tf^B$; fish with both bands are heterozygotes with genotype $Tf^A/Tf^B$.

This interpretation is based on the same kinds of evidence as led to a similar conclusion about plasma esterase. First, physical appearance of the marlin transferrin patterns is similar to that of chimpanzees (Goodman and Riopelle, 1963), rhesus monkeys (Goodman and Wolf, 1963), deer mice (Rasmussen and Koehn, 1966), humans (Seppala, Ruoslahti, and Makela, 1967), and marsupial mice (Hope and Godfrey, 1968). Breeding studies or family data have shown that electrophoretically distinct forms of transferrin are inherited in this manner in these species. Second, our data from different geographical areas conform to the Hardy-Weinberg model (table 3). Third, transferrin patterns are unrelated to sex and size of the fish (table 4). Moreover, as with plasma esterase, there is no apparent relation between transferrin variations and the reproductive cycle.

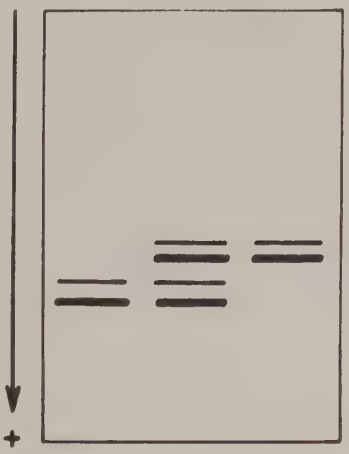


FIGURE 2.—Electrophoretic patterns of transferrin showing, from left to right, phenotypes Tf-A, Tf-AB, and Tf-B.

TABLE 3.—*Incidence of transferrin variants in different geographical areas, with comparison between observed distributions of phenotypes and (in parentheses) theoretical distributions based on inheritance controlled by two codominant alleles*

| Area | Number of fish | Phenotype | | | p [1] | $\chi^2$ | Probability df=1 |
|---|---|---|---|---|---|---|---|
| | | Tf-A | Tf-AB | Tf-B | | | |
| Middle Atlantic Bight | 152 | 89 (91.5) | 58 (52.7) | 5 (7.6) | 0.776 | 1.49 | 0.25>P>0.10 |
| Florida Straits | 95 | 56 (53.7) | 31 (35.3) | 8 (6.0) | 0.752 | 1.29 | 0.50>P>0.25 |
| Northern Gulf of Mexico | 70 | 51 (49.6) | 16 (18.6) | 3 (1.7) | 0.842 | 1.40 | 0.25>P>0.10 |
| Lower Caribbean Sea | 32 | 18 (17.2) | 11 (12.5) | 3 (2.2) | 0.734 | 0.35 | 0.75>P>0.50 |
| Total | 349 | 214 (211.5) | 116 120.1) | 19 (16.8) | 0.779 | 0.46 | 0.50>P>0.25 |

[1] Frequency of allele *Tf*

TABLE 4.—*Contingency test for independence of transferrin phenotypes and sex and size of fish*

| | Frequency of phenotypes [1] | | | $\chi^2$ | df | Probability |
|---|---|---|---|---|---|---|
| | Tf-A | Tf-AB | Tf-B | | | |
| Sex: | | | | | | |
| Male | 75(72.8) | 34(37.0) | 8(7.2) | 0.71 | 2 | 0.75>P>0.50 |
| Female | 98(100.2) | 54(51.0) | 9(9.8) | | | |
| Fork length (cm): [2] | | | | | | |
| ≤ 150.0 | 20(19.7) | 11(10.6) | 1(1.7) | | | |
| 150.1–160.0 | 76(77.6) | 42(41.6) | 8(6.9) | 3.82 | 6 | 0.75>P>0.50 |
| 160.1–170.0 | 55(56.0) | 33(30.0) | 3(5.0) | | | |
| ≥ 170.1 | 30(27.7) | 11(14.8) | 4(2.4) | | | |

[1] Number of fish with each phenotype actually observed and (in parentheses) expected numbers, calculated from row and column totals.
[2] Here defined as distance from tip of lower jaw to fork of caudal fin.

Es–1 and transferrin phenotypes are independent of each other (contingency chi-square=5.62, df=4, $0.25>P>0.10$). That is, the frequency distribution of transferrin phenotypes is about the same for each Es–1 phenotype. Since in this case phenotypes seem equivalent to genotypes, Es–1 and transferrin genotypes apparently are independent of each other. This apparent independence suggests that genetic loci for Es–1 and transferrin in white marlin are either widely separated on the same chromosome or occupy different chromosomes.

### 6-phosphogluconate dehydrogenase

This enzyme from white marlin RBC hemolysates occurs in five electrophoretically distinct forms. The band farthest from the origin is here designated PGD-A, the band nearest to the origin is designated PGD-C, and the band midway between them is designated PGD-B.

Most fish have only band B. Some fish have bands A and B with a more intensely stained band midway between them. Others have bands B and C with a more intensely stained band midway between them. Two fish have only band C. All white marlin that we have examined thus exhibit one of four PGD phenotypes (fig. 3).

I believe that these individual differences result from segregation of three codominant autosomal alleles, here designated $PGD^A$, $PGD^B$, and $PGD^C$. Fish with only band B, according to this in-

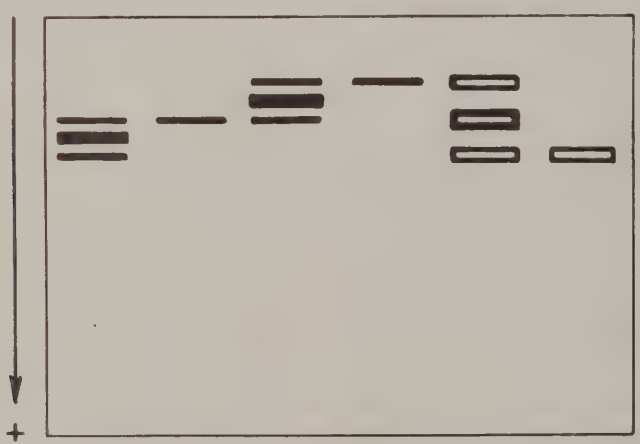


FIGURE 3.—Electrophoretic patterns of 6-phosphogluconate dehydrogenase from RBC showing, from left to right, phenotypes PGD-AB, -B, -BC, -C, -AC, and -A; the last two are hypothetical patterns not actually observed.

terpretation, are homozygotes with genotype $PGD^B/PGD^B$; fish with only band C are homozygotes with genotype $PGD^C/PGD^C$; fish with bands A, B, and their intermediate are heterozygotes with genotype $PGD^A/PGD^B$; and fish with bands B, C, and their intermediate are heterozygotes with genotype $PGD^B/PGD^C$. Intensely stained intermediate bands found only in presumed heterozygotes are probably hybrid molecules containing one unit each of PGD-A and PGD-B or one unit each of PGD-B and PGD-C (Shaw, 1965). Since heterozygotes with either PGD-A or PGD-C are uncommon, heterozygotes with both PGD-A and PGD-C must be very rare. $PGD^A$ homozygotes and $PGD^C$ homozygotes also must be rare. In our samples comprising 118 marlin, occurrence of only two $PGD^C$ homozygotes and absence of $PGD^A$ homozygotes and $PGD^A/PGD^C$ heterozygotes are therefore not surprising.

This interpretation of white marlin PGD variations rests on two kinds of evidence. First, the electrophoretic patterns are similar to those of humans (Fields and Parr, 1963), rats (Parr, 1966), cats (Thuline, Morrow and Motulsky, 1967), and pigs (Saison and Giblett, 1969). Family data or breeding studies prove that PGD variations in these mammals are controlled by codominant autosomal alleles. Second, the apparently heterozygous condition ($PGD^A/PGD^B$ and $PGD^B/PGD^C$) occurs in both males and females. Although this fact does not prove that PGD variations in white marlin are autosomally inherited, it eliminates the possibility of sex-linked inheritance.

PGD from fish seems to be an easily degraded enzyme (S. Ohno, personal communication). In white marlin it is less stable than esterase or transferrin. Very little PGD activity remains in homogenates made from tissues that had been frozen for 3 months, and identification of PGD phenotypes is impossible because the bands are badly smudged. The practical significance of these facts is that PGD analyses must be done promptly with fresh RBC hemolysates, whereas esterase and transferrin analyses can be carried out after plasmas have been frozen for several months. We have not analyzed fresh tissues for PGD. To work exclusively with fresh material from large numbers of fish needed in population surveys is impractical because preparation of tissue homogenates is time-consuming.

### *Malate dehydrogenase*

This enzyme occurs in white marlin tissues in three major electrophoretically distinct forms. The form farthest from the origin is here designated MDH-A, and the form nearest the origin is designated MDH-B. Up to four diffuse bands anodal to band A and between bands A and B are more evident on some gels than on others.

We analyzed heart homogenates from 168 marlin and RBC hemolysates from an additional 103 marlin. Three of these individuals had MDH-A and MDH-B plus a more intensely stained band midway between them. All other fish had only MDH-B (fig. 4).

I propose that these individual differences result from segregation of two codominant alleles, here designated $MDH^A$ and $MDH^B$. According to this interpretation, fish with only MDH-B are homozygotes with genotype $MDH^B/MDH^B$; fish with both MDH-A and MDH-B plus the intermediate band are heterozygotes with genotype $MDH^A/MDH^B$. The intermediate band is probably a hybrid molecule containing one unit each of MDH-A and MDH-B. Bailey, Cocks, and Wilson (1969) found

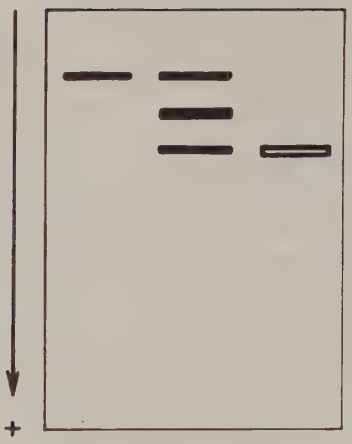


FIGURE 4.—Electrophoretic patterns of malate dehydrogenase from RBC showing, from left to right, phenotypes MDH-B, MDH-AB, and MDH-A; the last is a hypothetical pattern not actually observed.

hybrid MDH molecules of this kind in rainbow trout and red salmon and showed that electrophoretic variants of MDH in these species are in fact under genetic control. Their work lends credibility to the view that MDH variations among white marlin are hereditary.

It is uncertain whether the proposed inheritance of MDH in white marlin is sex-linked or autosomal. Two of the three marlin presumed heterozygous for MDH are females. Sex of the third is unknown because I did not dissect the fish. In general, occurrence of the heterozygous condition only in females indicates sex-linked inheritance. However, in the case of white marlin, additional heterozygotes must be identified and classified by sex before a sound inference can be made on this matter.

MDH patterns in white marlin are apparently not tissue specific. Homogenates of muscle, heart, liver, and gonads and RBC hemolysates have virtually identical electrophoretic patterns except that RBC show little or no subbanding. In this respect, white marlin differ from the cyprinodontiform fish *Oryzias latipes*, in which, according to Nakano and Whitely (1965), MDH patterns are tissue specific.

### *Lactate dehydrogenase*

This enzyme occurs in white marlin skeletal muscle tissue in four major electrophoretically distinct forms (fig. 5).

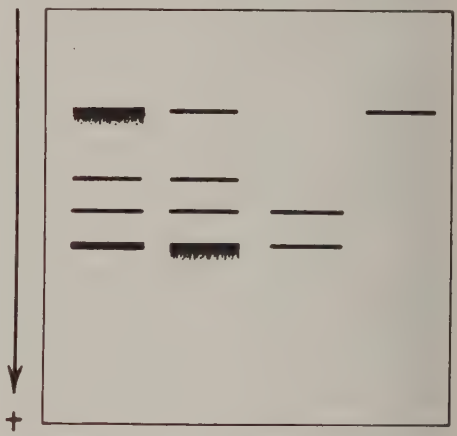


FIGURE 5.—Electrophoretic patterns of lactate dehydrogenase showing, from left to right, tissue specific patterns from skeletal muscle, heart muscle (liver, ovary, and testis are similar to heart), blood plasma, and RBC.

In keeping with conventional LDH nomenclature, I designate the band farthest from the origin LDH–1 and the others, in order of decreasing electrophoretic mobility, LDH–2, LDH–3, and LDH–5. White marlin lack LDH–4, which is detectable in numerous vertebrate species as a band midway between LDH–3 and LDH–5 (Markert, 1968); Markert stated that some fish and reptiles lack LDH–4 but listed only one example, the snake *Dryodophis melanolomus*. In having only four major LDH bands white marlin differ from fluke (summer flounder) with only one; from porgy (scup), Atlantic and Spanish mackerels, gray snapper, and striped mullet with two; from weakfish, butterfish, striped bass, and

spot with three; and from shad, herring, and silver hake with five (Markert and Faulhaber, 1965). Minor bands in the vicinity of LDH–1 are present on some gels but absent from others. Markert (1968) asserted that minor LDH bands probably reflect subtle variations in the arrangement of the enzyme's constituent subunits, but the functional significance of minor bands is unexplained.

LDH patterns in white marlin are tissue specific; staining intensity of the bands is not the same for all tissues. LDH–1 predominates in heart, liver, testis, ovary, and blood plasma, staining far more intensely in heart homogenates than in other tissues. LDH–5 predominates in skeletal muscle and RBC. Markert and Faulhaber (1965) asserted that LDH–1 and LDH–5 predominate in mammalian heart and in skeletal muscle, respectively. They found a similar generalization about fishes is not justified because tissue specific patterns vary from one species to another.

We carried out LDH analyses on skeletal muscle homogenates from 145 white marlin but examined only about a dozen RBC hemolysates and plasmas. There were no variations among individual fish comparable to those of Es–1, transferrin, PGD, and MDH.

### *Glucose-6-phosphate dehydrogenase*

This enzyme is undetectable in homogenates made from skeletal muscle, heart, and gonad tissues that had been frozen for several months and in hemolysates made from 2-week-old RBC. But it appears on electrophoretic separations of liver homogenates from 168 fish as a diffuse zone about 5 mm wide. Some fish appear to have two bands within the zone but others appear to have only one. This distinction is a highly subjective one because the bands are close together and staining intensity of the bands varies among individual fish. Consequently I think the individual variations in G6PD that we detected—even if they are genetically controlled—will be of no value in white marlin subpopulation studies.

### *Hemoglobin*

This protein occurs in white marlin RBC in six electrophoretically distinct forms. The bands are equidistant but of unequal intensity; the second and fifth are prominent (fig. 6).

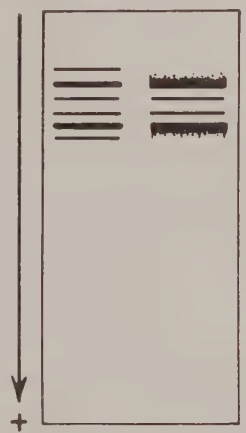


FIGURE 6.—Electrophoretic patterns of hemoglobin from fresh RBC (on the left) and 21-day-old RBC (on the right).

Fish hemoglobin is generally unstable and deteriorates quickly (Vanstone, Roberts, and Tsuyuki, 1964; Sick, 1965; Wilkins and Iles, 1966). Nevertheless, storage of untreated marlin blood samples at 5°C for up to 24 days produces only minor changes in hemoglobin patterns; leading and trailing bands are faint or undetectable and the other four bands are slightly more diffuse. These changes are apparently unrelated to the duration of storage; electrophoretic patterns of 2-day-old and 24-day-old material are indistinguishable from each other although both differ slightly from those of fresh RBC. Analyses of blood samples from 335 white marlin revealed no individual

variations other than those attributable to sample age.

## DISCUSSION

Electrophoresis has been used before as a means of estimating the amount of genic variability in natural populations. Lewontin and Hubby (1966) found genic variants in seven of 18 arbitrarily selected proteins from fruit flies. Harris (1966) reported that of 10 randomly chosen enzyme proteins from human blood, two exhibit heritable variation. Ruddle et al. (1969) detected polymorphism at 5 of 17 genetic loci that control enzymes in the house mouse. Incidence of genic polymorphism in white marlin seems even higher than in these species; four of seven proteins that we surveyed exist as genic variants that are distinguishable by electrophoretic techniques.

Using these techniques we classified 16 lots of white marlin from four widely separated geographical areas according to Es–1 and transferrin phenotypes (tables 1 and 3, respectively). Within each area there is good agreement between the observed distributions of phenotypes and the Hardy-Weinberg binomial distributions based on two-allele inheritance. This both strengthens the case for genetic control of Es–1 and transferrin variations and suggests that lots were collected from a genetically homogeneous population in each area. Moreover, the observed distribution of Es–1 phenotypes and the observed distribution of transferrin phenotypes in the Middle Atlantic Bight are very similar to those in the Florida Straits, the Gulf of Mexico and the southern Caribbean Sea (contingency chi-square for Es–1=1.66, df=6, $0.95>P>0.90$; contingency chi-square for transferrin=9.27, df=6, $0.25>P>0.10$). This similarity means that Es–1 and transferrin systems as yet provide no evidence to justify assigning marlin from these areas to different subpopulations. Nor do the geographical distributions of PGD variants (table 5) or MDH variants provide evidence of subpopulations in the western North Atlantic.

There are three possible explanations for our failure to detect white marlin subpopulations in the western North Atlantic. One is that we have Es–1, transferrin, PGD, and MDH data from too few fish. Another is that comparisons based on these four systems will not differentiate subpopulations regardless of the number of fish examined. Comparisons based on genetically controlled serum esterase variants differentiate a skipjack tuna subpopulation in the central Pacific from another in the western Pacific whereas comparisons based on genetically controlled transferrin variants do not (Fujino and Kang, 1968a, 1968b). In this sense Es–1, transferrin, PGD, and MDH may be the "wrong" characters to reveal white marlin subpopulations. A third explanation is that subpopulations of white marlin do not exist in the western North Atlantic. All fish present during the summer in the Middle Atlantic Bight, in the northern Gulf

TABLE 5.—*Incidence of PGD variants in different geographical areas*

[I did not calculate chi-square for each area because the few fish with phenotype PGD-AB or -BC do not justify doing so. Consequently there is no statistical test of the hypothesis of heredity control by three codominant alleles. It is clear that phenotypic proportions in the three areas are similar.]

| Area | Number of fish | Phenotype PGD | | | | | |
|---|---|---|---|---|---|---|---|
| | | -A | -AB | -B | -BC | -C | -AC |
| Middle Atlantic Bight | 64 | 0 | 4 | 52 | 7 | 1 | 0 |
| Florida Straits | 32 | 0 | 1 | 27 | 3 | 1 | 0 |
| Lower Caribbean Sea | 22 | 0 | 2 | 19 | 1 | 0 | 0 |
| Total | 118 | 0 | 7 | 98 | 11 | 2 | 0 |

of Mexico, and in the lower Caribbean Sea may belong to a single homogeneous breeding population. In that case the incidence of any genetically controlled variant would be the same in these areas.

I think the third explanation is the most plausible. It is consistent with evidence from tagging, which shows that white marlin migrate between widely separated areas in the western North Atlantic but apparently do not cross the equator into the South Atlantic (Mather, 1967; F. J. Mather, III, A. C. Jones, and G. L. Beardsley, Jr., unpublished). It is also consistent with information about spawning activity. De Sylva and Davis (1963) reported that female white marlin landed in the Bahamas during the spring had ripening ovaries but females landed at Ocean City, Md., during the summer did not. I confirmed their observations. Moreover all the white marlin that I examined during the summer in the northern Gulf of Mexico and in Venezuela contained ovaries that were reproductively inactive—turgid, flesh-colored, and without visible ova. The few fish landed each year by sportsmen in Venezuela between November and May are also reproductively undeveloped (R. Jaen C., personal communication). These facts suggest that white marlin spawn during May and June in the western North Atlantic and that, as de Sylva and Davis believed, spawning occurs between the Bahamas and the mid-Atlantic coast of the United States.

There is no conclusive evidence of spawning elsewhere in the North Atlantic. Japanese scientists collected one billfish larva, tentatively identified as white marlin, at each of three locations in the subtropical North Atlantic during January of 1967 (S. Ueyanagi, personal communication). But spawning in the area has not been confirmed by finding either ripe adults or postlarvae and juveniles that could be positively identified.

White marlin also spawn south of the equator. In February and March of 1966 Japanese scientists collected both ripe adults and larvae, the latter tentatively identified as white marlin, throughout a broad area in the central and western South Atlantic between 15° and 25°S. latitude. They also collected a single larva in January of 1967 about 40 miles northeast of Recife, Brazil (S. Ueyanagi, personal communication). Since there is apparently no emigration to these areas from the North Atlantic, the South Atlantic spawning probably represents a second discrete breeding population.

In summary, there is presumptive evidence white marlin in the western North Atlantic belong to a single homogeneous breeding population that spawns during May and June between the Bahamas and the mid-Atlantic coast of the United States. There is conclusive proof white marlin spawn in the South Atlantic several hundred miles off the Brazilian coast during February and March. They apparently do not migrate back and forth across the equator between these areas. I infer that there are at least two subpopulations of white marlin: one in the western North Atlantic and one or more in the South Atlantic.

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